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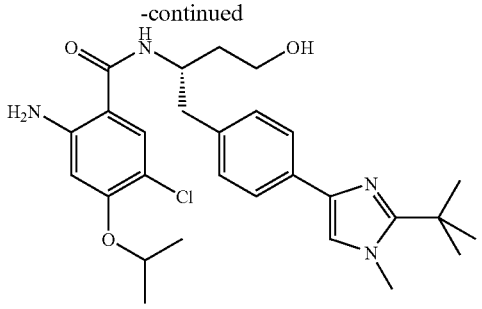
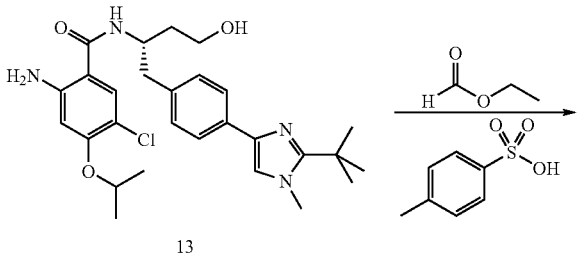


13

[1026] The procedure of taking compound 6 to compound 8 in Example 100 was followed for making 11.

[1027] To a solution of crude 11 (160 mg, 0.97 mmol) in DMF (1 mL) were successively added compound 12 (235 mg, 0.97 mmol), HBTU (396 mg, 1.05 mmol) and DIEA (606 μL, 3.48 mmol). The solution was stirred at room temperature for 1 hour and purified by RP-HPLC using a mixture of acetonitrile and H_2O to give 13 (100 mg, 20%). LRMS ($M+H^+$) m/z 513.2.



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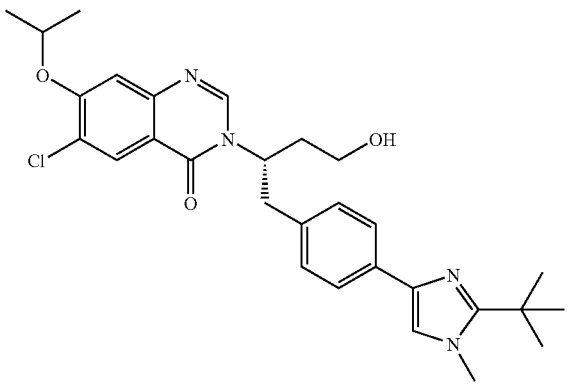

14

[1028] Compound 13 (37 mg, 0.072 mmol), ethyl formate (2 mL) and p-toluene sulfonic acid (4 mg) were mixed in the closed tube and heated to 140° C. for about 8 hours. The solution was concentrated and purified by RP-HPLC using a mixture of acetonitrile and H_2O to give 14 (10 mg, 27%). LRMS ($M+H^+$) m/z 523.2.

Example 125

Inhibition of Cellular Viability in Tumor Cell Lines Treated with Mitotic Kinesin Inhibitors

[1029] Materials and Solutions:

[1030] Cells: SKOV3, Ovarian Cancer (human).

[1031] Media: Phenol Red Free RPMI+5% Fetal Bovine Serum+2 mM L-glutamine.

[1032] Colorimetric Agent for Determining Cell Viability: Promega MTS tetrazolium compound.

[1033] Control Compound for max cell kill: Topotecan, 1 μM.

[1034] Procedure: Day 1—Cell Plating:

[1035] Adherent SKOV3 cells are washed with 10 mLs of PBS followed by the addition of 2 mLs of 0.25% trypsin and incubation for 5 minutes at 37° C. The cells are rinsed from the flask using 8 mL of media (phenol red-free RPMI+5% FBS) and transferred to fresh flask. Cell concentration is determined using a Coulter counter and the appropriate volume of cells to achieve 1000 cells/100 μL is calculated. 100 μL of media cell suspension (adjusted to 1000 cells/100 μL) is added to all wells of 96-well plates, followed by incubation for 18 to 24 hours at 37° C., 100% humidity, and 5% CO_2, allowing the cells to adhere to the plates.

[1036] Procedure: Day 2—Compound Addition:

[1037] To one column of the wells of an autoclaved assay block are added an initial 2.5 μL of test compound(S) at 400× the highest desired concentration. 1.25 μL of 400× (400 μM) Topotecan is added to other wells (ODs from these wells are used to subtract out for background absorbance of dead cells and vehicle). 500 μL of media without DMSO are added to the wells containing test compound, and 250 μL to the Topotecan wells. 250 μL of media+0.5% DMSO is added to all remaining wells, into which the test compound(S) are serially diluted. By row, compound-containing media is replica plated (in duplicate) from the assay block to the corresponding cell plates. The cell plates are incubated for 72 hours at 37° C., 100% humidity, and 5% CO_2.

[1038] Procedure: Day 4—MTS Addition and OD Reading:

[1039] The plates are removed from the incubator and 40 μl MTS/PMS is added to each well. Plates are then incubated for 120 minutes at 37° C., 100% humidity, 5% CO_2, followed by reading the ODs at 490 nm after a 5 second shaking cycle in a ninety-six well spectrophotometer.

Data Analysis

[1040] The normalized % of control (absorbance−background) is calculated and an XLfit is used to generate a dose-response curve from which the concentration of compound required to inhibit viability by 50% is determined. The compounds of the present invention show activity when tested by this method.

Example 126

Application of a Mitotic Kinesin Inhibitor

[1041] Human tumor cells Skov-3 (ovarian) were plated in 96-well plates at densities of 4,000 cells per well, allowed to adhere for 24 hours, and treated with various concentrations of the test compounds for 24 hours. Cells were fixed in 4% formaldehyde and stained with antitubulin antibodies (subsequently recognized using fluorescently-labeled secondary antibody) and Hoechst dye (which stains DNA).